USP 24
NF 19

U.S. Pharmacopeia & National Formulary

2000

USP 24

NF 19

THE UNITED STATES PHARMACOPEIA

THE NATIONAL FORMULARY

By authority of the United States Pharmacopeial Convention, Inc., meeting at Washington, D.C., March 9–12, 1995. Prepared by the Committee of Revision and published by the Board of Trustees

Official from January 1, 2000

UNITED STATES PHARMACOPEIAL CONVENTION, INC.
12601 Twinbrook Parkway, Rockville, MD 20852

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ISSN 0195-7996
ISBN 1-889788-03-1

Printed by National Publishing, Philadelphia, PA

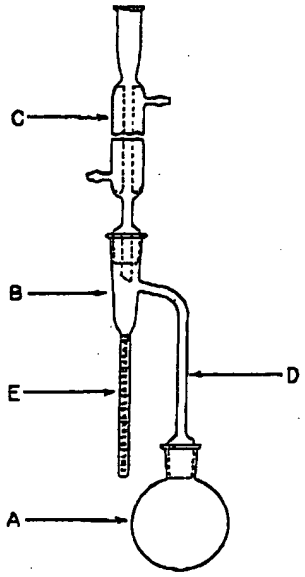
or as accurately prepared solutions in appropriate anhydrous solvents.

Where the specimen is an insoluble solid, the water may be extracted using a suitable anhydrous solvent from which an appropriate quantity, accurately weighed, may be injected into the anolyte solution. Alternatively an evaporation technique may be used in which water is released and evaporated by heating the specimen in a tube in a stream of dry inert gas, this gas being then passed into the cell.

Procedure—Using a dry syringe, quickly inject the *Test Preparation*, accurately measured and estimated to contain 0.5 to 5 mg of water, into the anolyte, mix, and perform the coulometric titration to the electrometric endpoint. Read the water content of the *Test Preparation* directly from the instrument's display, and calculate the percentage that is present in the substance. Perform a blank determination, and make any necessary corrections.

METHOD II (AZEOTROPIC—TOLUENE DISTILLATION)

Apparatus—Use a 500-mL glass flask *A* connected by means of a trap *B* to a reflux condenser *C* by ground glass joints (see figure).



Toluene Moisture Apparatus

The critical dimensions of the parts of the apparatus are as follows. The connecting tube *D* is 9 to 11 mm in internal diameter. The trap is 235 to 240 mm in length. The condenser, if of the straight-tube type, is approximately 400 mm in length and not less than 8 mm in bore diameter. The receiving tube *E* has a 5-mL capacity, and its cylindrical portion, 146 to 156 mm in length, is graduated in 0.1-mL subdivisions, so that the error of reading is not greater than 0.05 mL for any indicated volume. The source of heat is preferably an electric heater with rheostat control or an oil bath. The upper portion of the flask and the connecting tube may be insulated.

Clean the receiving tube and the condenser with chromic acid cleansing mixture, thoroughly rinse with water, and dry in an oven. Prepare the toluene to be used by first shaking with a small quantity of water, separating the excess water, and distilling the toluene.

Procedure—Place in the dry flask a quantity of the substance, weighed accurately to the nearest centigram, which is expected to yield 2 to 4 mL of water. If the substance is of a pasty character, weigh it in a boat of metal foil of a size that will just pass through the neck of the flask. If the substance is likely to cause bumping, add enough dry, washed sand to cover the bottom of the flask, or a number of capillary melting-point tubes, about 100 mm in length, sealed at the upper end. Place about 200 mL of toluene in the flask, connect the apparatus, and fill the receiving tube *E* with toluene poured through the top of the condenser. Heat the flask gently for 15 minutes and, when the toluene begins to boil, distil at the rate of about 2 drops per second until most of the water has passed over, then increase the rate of distillation to about 4 drops per second. When the water has apparently all distilled over, rinse the inside of the condenser tube with toluene while brushing down the tube with a tube brush attached to a copper wire and saturated with toluene. Continue the distillation for 5 minutes, then remove the heat, and allow the receiving tube to cool to room temperature. If any droplets of water adhere to the walls of the receiving tube, scrub them down with a brush consisting of a rubber band wrapped around a copper wire and wetted with toluene. When the water and toluene have separated completely, read the volume of water, and calculate the percentage that was present in the substance.

METHOD III (GRAVIMETRIC)

Procedure for Chemicals—Proceed as directed in the individual monograph preparing the chemical as directed under *Loss on Drying* ⟨731⟩.

Procedure for Biologics—Proceed as directed in the individual monograph.

Procedure for Vegetable Drugs—Place about 10 g of the drug, prepared as directed (see *Vegetable Drugs—Methods of Analysis* ⟨561⟩), and accurately weighed, in a tared evaporating dish. Dry at 105° for 5 hours, and weigh. Continue the drying and weighing at 1-hour intervals until the difference between two successive weighings corresponds to not more than 0.25%.

⟨941⟩ X-RAY DIFFRACTION

Every crystal form of a compound produces its own characteristic X-ray diffraction pattern. These diffraction patterns can be derived either from a single crystal or from a powdered specimen (containing numerous crystals) of the material. The spacings between and the relative intensities of the diffracted maxima can be used for qualitative and quantitative analysis of crystalline materials. Powder diffraction techniques are most commonly employed for routine identification and the determination of relative purity of crystalline materials. Small amounts of impurity, however, are not normally detectable by the X-ray diffraction method, and for quantitative measurements it is necessary to prepare the sample carefully to avoid preferred orientation effects.

The powder methods provide an advantage over other means of analysis in that they are usually nondestructive in nature (specimen preparation is usually limited to grinding to ensure a randomly oriented sample, and deleterious effects of X-rays on solid pharmaceutical compounds are not commonly encountered). The principal use of single-crystal diffraction data is for the determination of molecular weights and analysis of crystal structures at the atomic level. However, diffraction established for a single crystal can be used to support a specific powder pattern as being truly representative of a single phase.

Solids—A solid substance can be classified as being crystalline, noncrystalline, or a mixture of the two forms. In crystalline materials, the molecular or atomic species are ordered in a three-dimensional array, called a lattice, within the solid particles. This ordering of molecular components is lacking in noncrystalline material. Noncrystalline solids sometimes are referred to as glasses or amorphous solids when repetitive order is nonexistent in all three dimensions. It is also possible for order to exist in only one or two dimensions, resulting in mesomorphic phases (liquid crystals). Although crystalline materials are usually considered to have well-defined visible external morphologies (their habits), this is not a necessity for X-ray diffraction analysis.

The relatively random arrangement of molecules in noncrystalline substances makes them poor coherent scatterers of X-rays, resulting in broad, diffuse maxima in diffraction patterns. Their X-

ray patterns are quite distinguishable from crystalline specimens, which give sharply defined diffraction patterns.

Many compounds are capable of crystallizing in more than one type of crystal lattice. At any particular temperature and pressure, only one crystalline form (polymorph) is thermodynamically stable. Since the rate of phase transformation of a metastable polymorph to the stable one can be quite slow, it is not uncommon to find several polymorphs of crystalline pharmaceutical compounds existing under normal handling conditions.

In addition to exhibiting polymorphism, many compounds form crystalline solvates in which the solvent molecule is an integral part of the crystal structure. Just as every polymorph has its own characteristic X-ray patterns, so does every solvate. Sometimes the differences in the diffraction patterns of different polymorphs are relatively minor, and must be very carefully evaluated before a definitive conclusion is reached. In some instances, these polymorphs and / or solvates show varying dissolution rates. Therefore, on the time scale of pharmaceutical bioavailability, different total amounts of drug are dissolved, resulting in potential bioinequivalence of the several forms of the drug.

Fundamental Principles—A collimated beam of monochromatic X-rays is diffracted in various directions when it impinges upon a rotating crystal or randomly oriented powdered crystal. The crystal acts as a three-dimensional diffraction grating to this radiation. This phenomenon is described by Bragg's law, which states that diffraction (constructive interference) can occur only when waves that are scattered from different regions of the crystal, in a specific direction, travel distances differing by integral numbers (n) of the wavelength (λ). Under such circumstances, the waves are in phase. This condition is described by the Bragg equation:

$$\frac{n\lambda}{2 \sin \theta} = d_{hkl},$$

in which d_{hkl} denotes the interplanar spacings and θ is the angle of diffraction.

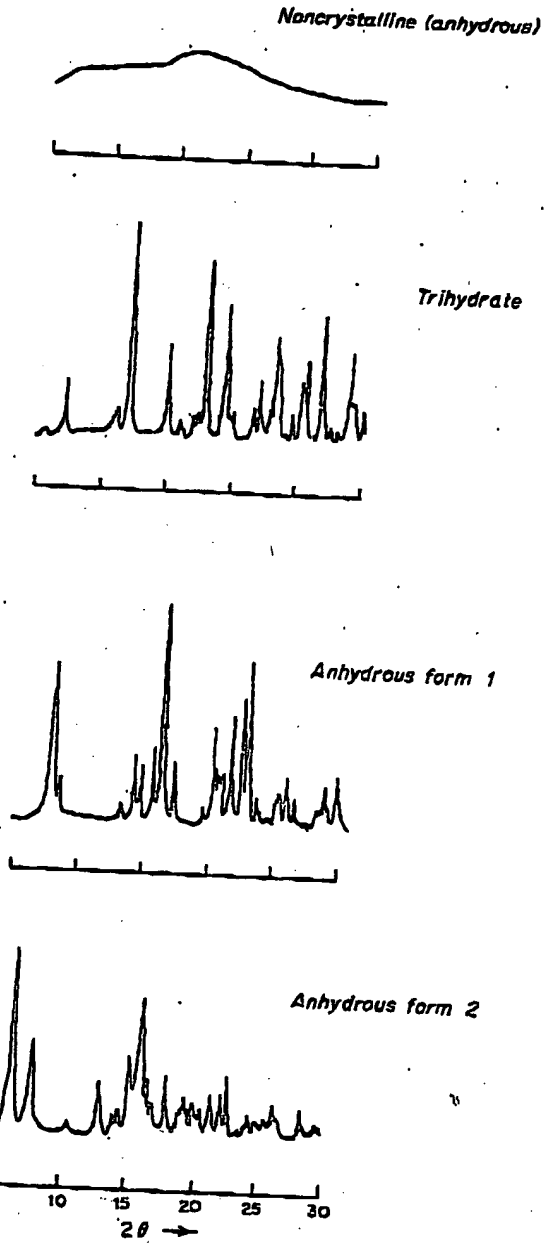
A family of planes in space can be indexed by three whole numbers, usually referred to as Miller indices. These indices are the reciprocals, reduced to smallest integers, of the intercepts that a plane makes along the axes corresponding to three nonparallel edges of the unit cell (basic crystallographic unit). The unit cell dimensions are given by the lengths of the spacings along the three axes, a, b, c, and the angles between them, α, β, and γ. The interplanar spacing for a specific set of parallel planes hkl is denoted by d_{hkl}. Each such family of planes may show higher orders of diffraction where the d values for the related families of planes nh, nk, nl are diminished by the factor $1/n$ (n being an integer: 2, 3, 4, etc.). Every set of planes throughout a crystal has a corresponding Bragg diffraction angle associated with it (for a specific λ).

The amplitude of a diffracted X-ray beam from any set of planes is dependent upon the following atomic properties of the crystal: (1) position of each atom in the unit cell; (2) the respective atomic scattering factors; and (3) the individual thermal motions. Other factors that directly influence the intensities of the diffracted beam are: (1) the intensity and wavelength of the incident radiation; (2) the volume of crystalline specimen; (3) the absorption of the X-radiation by the specimen; and (4) the experimental arrangement utilized to record the intensity data. Thus, the experimental conditions are especially important for measurement of diffraction intensities.

Only a limited number of Bragg planes are in a position to diffract when monochromatized X-rays pass through a single crystal. Techniques of recording the intensities of all of the possible diffracting hkl planes involve motion of the single crystal and the recording media. Recording of these data is accomplished by photographic techniques (film) or with radiation detectors.

A beam passing through a very large number of small, randomly oriented crystals produces continuous cones of diffracted rays from each set of lattice planes. Each cone corresponds to the diffraction from various planes having a similar interplanar spacing. The intensities of these Bragg reflections are recorded by either film or radiation detectors. The Bragg angle can be measured easily from a film, but the advent of radiation detectors has made possible the construction of diffractometers that read this angle directly. The intensities and d spacings are more conveniently determined with powder diffractometers employing radiation detectors than by film methods. Microphotometers are frequently used for precise intensity measurements of films.

An example of the type of powder patterns obtained for four different solid phases of ampicillin are shown in the accompanying figure. These diffraction patterns were derived from a powder diffractometer equipped with a Geiger-Müller detector; nickel-filtered Cu $K\alpha$ radiation was used.



Typical Powder Patterns Obtained for Four Solid Phases of Ampicillin

Radiation—The principal radiation sources utilized for X-ray diffraction are vacuum tubes utilizing copper, molybdenum, iron, and chromium as anodes; copper X-rays are employed most commonly for organic substances. For each of these radiations there is an element that will filter off the $K\beta$ radiation and permit the $K\alpha$ radiation to pass (nickel is used, in the case of copper radiation). In this manner the radiation is practically monochromatized. The choice of radiation to be used depends upon the absorption characteristics of the material and possible fluorescence by atoms present in the specimen.

Caution—Care must be taken in the use of such radiation. Those not familiar with the use of X-ray equipment should seek expert advice. Improper use can result in harmful effects to the operator.

Test Preparation—In an attempt to improve randomness in the orientation of crystallites (and, for film techniques, to avoid a grainy pattern), the specimen may be ground in a mortar to a fine powder. Grinding pressure has been known to induce phase transformations; therefore, it is advisable to check the diffraction pattern of the unground sample.

In general, the shapes of many crystalline particles tend to give a specimen that exhibits some degree of preferred orientation in the specimen holder. This is especially evident for needle-like or plate-like crystals where size reduction yields finer needles or platelets. Preferred orientation in the specimen influences the relative intensities of various reflections.

Several specialized handling techniques may be employed to minimize preferred orientation, but further reduction of particle size is often the best approach.

Where very accurate measurement of the Bragg angles is necessary, a small amount of an internal standard can be mixed into the specimen. This enables the film or recorder tracing to be calibrated. If comparisons to literature values (including compendial limits) of *d* are being made, calibrate the diffractometer. NIST standards are available covering to a *d*-value of 0.998 nm. Tetradecanol[1] may be used (*d* is 3.963 nm) for larger spacing.

The absorption of the radiation by any specimen is determined by the number and kinds of atoms through which the X-ray beam passes. An organic matrix usually absorbs less of the diffracted radiation than does an inorganic matrix. Therefore, it is important in quantitative studies that standard curves relating amount of material to the intensity of certain *d* spacings for that substance be determined in a matrix similar to that in which the substance will be analyzed.

In quantitative analyses of materials, a known amount of standard usually is added to a weighed amount of specimen to be analyzed. This enables the amount of the substance to be determined relative to the amount of standard added. The standard used should have approximately the same density as the specimen and similar absorption characteristics. More important, its diffraction pattern should not overlap to any extent with that of the material to be analyzed. Under these conditions a linear relationship between line intensity and concentration exists. In favorable cases, amounts of crystalline materials as small as 10% may be determined in solid matrices.

Identification of crystalline materials can be accomplished by comparison of X-ray powder diffraction patterns obtained for known[2] materials with those of the unknown. The intensity ratio (ratio of the peak intensity of a particular *d* spacing to the intensity of the strongest maxima in the diffraction pattern) and the *d* spacing are used in the comparison. If a reference material (e.g., USP Reference Standard) is available, it is preferable to generate a primary reference pattern on the same equipment used for running the unknown sample, and under the same conditions. For most organic crystals, it is appropriate to record the diffraction pattern to include values for 2θ that range from as near zero degrees as possible to 40 degrees. Agreement between sample and reference should be within the calibrated precision of the diffractometer for diffraction angle (2θ values should typically be reproducible to ±0.10 or 0.20 degrees), while relative intensities between sample and reference may vary considerably. For other types of samples (e.g., inorganic salts), it may be necessary to extend the 2θ region scanned to well beyond 40 degrees. It is generally sufficient to scan past the ten strongest reflections identified in the Powder Diffraction File.[3]

[1] Brindley, GW and Brown, G, eds., *Crystal Structures of Clay Minerals and their X-ray Identification*, Mineralogical Society Monograph No. 5, London, 1980, pp. 318 ff.

[3] The International Centre for Diffraction Data, Newtown Square Corporate Campus, 12 Campus Boulevard, Newtown Square, PA 19073, maintains a file on more than 60,000 crystalline materials, both organic and inorganic, suitable for such comparisons.